# Endogenous -calcitonin-gene-related peptide promotes exercise-induced, physiological heart hypertrophy in mice

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Abstract: AIM: It is unknown how the heart distinguishes various overloads, such as exercise or hypertension, causing either physiological or pathological hypertrophy. We hypothesize that alpha-calcitonin-gene-related peptide ( CGRP), known to be released from contracting skeletal muscles, is key at this remodelling. METHODS: The hypertrophic effect of CGRP was measured in vitro (cultured cardiac myocytes) and in vivo (magnetic resonance imaging) in mice. Exercise performance was assessed by determination of maximum oxygen consumption and time to exhaustion. Cardiac phenotype was defined by transcriptional analysis, cardiac histology and morphometry. Finally, we measured spontaneous activity, body fat content, blood volume, haemoglobin mass and skeletal muscle capillarization and fibre composition. RESULTS: While CGRP exposure yielded larger cultured cardiac myocytes, exercise-induced heart hypertrophy was completely abrogated by treatment with the peptide antagonist CGRP(8-37). Exercise performance was attenuated in CGRP(-/-) mice or CGRP(8-37) treated wild-type mice but improved in animals with higher density of cardiac CGRP receptors (CLR-tg). Spontaneous activity, body fat content, blood volume, haemoglobin mass, muscle capillarization and fibre composition were unaffected, whereas heart index and ventricular myocyte volume were reduced in CGRP(-/-) mice and elevated in CLR-tg. Transcriptional changes seen in CGRP(-/-) (but not CLR-tg) hearts resembled maladaptive cardiac phenotype. CONCLUSIONS: Alpha-calcitonin-gene-related peptide released by skeletal muscles during exercise is a hitherto unrecognized effector directing the strained heart into physiological instead of pathological adaptation. Thus, CGRP agonists might be beneficial in heart failure patients.

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# Endogenous α-Calcitonin gene-related peptide promotes exercise-induced, physiological heart hypertrophy in mice

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**Abstract**

*Aim:* It is unknown how the heart distinguishes various overloads, such as exercise or hypertension, causing either physiological or pathological hypertrophy. We hypothesize that alpha calcitonin gene-related peptide (αCGRP), known to be released from contracting skeletal muscles, is key at this remodeling.

*Methods:* The hypertrophic effect of αCGRP was measured *in vitro* (cultured cardiac myocytes) and *in vivo* (magnetic resonance imaging) in mice. Exercise performance was assessed by determination of maximum oxygen consumption and time to exhaustion. Cardiac phenotype was defined by transcriptional analysis, cardiac histology and morphometry. Finally, we measured spontaneous activity, body fat content, blood volume, hemoglobin mass and skeletal muscle capillarization and fiber composition.

*Results:* While αCGRP exposure yielded larger cultured cardiac myocytes exercise-induced heart hypertrophy was completely abrogated by treatment with the peptide antagonist CGRP(8-37). Exercise performance was attenuated in $\alpha CGRP^{-/-}$ mice or CGRP(8-37) treated wild type mice but improved in animals with higher density of cardiac CGRP receptors (CLR-tg). Spontaneous activity, body fat content, blood volume, hemoglobin mass, muscle capillarization and fiber composition were unaffected whereas heart index and ventricular myocyte volume were reduced in $\alpha CGRP^{-/-}$ mice and elevated in CLR-tg. Transcriptional changes seen in $\alpha CGRP^{-/-}$ (but not CLR-tg) hearts resembled maladaptive cardiac phenotype.

*Conclusions:* αCGRP released by skeletal muscles during exercise is a hitherto unrecognized effector directing the strained heart into physiological instead of pathological adaptation. Thus, αCGRP agonists might be beneficial in heart failure patients.

**Keywords**

athlete's heart, cardiac hypertrophy, doping, endurance capacity, exercise performance, muscle metaboreflex, sport

**Introduction**

A long-standing question is why the heart adapts to different overloads by committing itself to either physiological or pathological hypertrophy. A widely believed, though never proven, tenet is that intermittent stimuli, such as exercise, promote physiological hypertrophy. In contrast, chronic stresses such as hypertension are thought to always induce pathological hypertrophy. However, according to a recent study (Perrino et al., 2006) it is the nature of the overload - independent of its chronicity - that determines the cardiac phenotype.

Adequate physical performance depends on the type, intensity and frequency of previous exercise that affects entire organs, blood vessels as well as sub-cellular structures (Hoppeler and Weibel, 1998). During exercise, acute nutrient and oxygen delivery to skeletal muscles is controlled by the sympathetic nervous system and local mechanisms based on proton, lactate, potassium and $CO_2$ release (Boushel, 2010). Since about 50% of maximum exercise capacity depends on cardiac output (Q) (Andersen and Saltin, 1985), among the adaptive mechanisms activated with endurance training is physiological, exercise-induced heart hypertrophy (i.e. "athlete's heart"). While the specific mechanisms that drive physiological myocardial hypertrophy in response to exercise are unknown they appear to be distinct from those of pathological hypertrophy - even when resulting in the same increase of ventricular mass (Perrino et al., 2006). Physiological heart hypertrophy could partly rest on a feedback loop, known as "metaboreflex", that adjusts the circulation to the needs of the exercising skeletal muscles by monitoring their activity and metabolic state through mechanoreceptors (group III or A$\partial$ fibers) and chemoreceptors (group IV or non-myelinated C fibers) (Boushel, Mitchell et al., 1983, Amann et al., 2011). Activated A$\partial$ and C fibers release αCGRP (Schmelz and Petersen, 2001), the plasma levels of αCGRP correlate tightly with exercise load (Schifter et al., 1995, Lind et al., 1996, Hasbak et al., 2002) and αCGRP binding sites have been detected throughout the heart (Sigrist et al., 1986). Whereas αCGRP generally causes positive inotropy on atria (Ishikawa et al., 1988) some authors (Huang et al., 1999) but not others (Ishikawa et al., 1988) found positive inotropic effects of the peptide on the ventricles. Moreover,

culturing cardiac myocytes in the presence of CGRP promotes hypertrophy of these cells (Bell et al., 1995, Bell et al., 1997). *In vivo*, systemic αCGRP might exert positive inotropic effects also by activating directly sympathetic postganglionic neurons (Katori et al., 2005) similar as described for αCGRP's positive chronotropic action (Kunz et al., 2007, Fisher et al., 1983).

The localization of αCGRP receptors, the positive inotropy of αCGRP on atria and maybe also on ventricles as well as the fact that increasing plasma levels of this peptide correlate specifically with the physical strain applied (Schifter et al., 1995, Lind et al., 1996, Hasbak et al., 2002) are all in line with the notion of exercising skeletal muscles might acting as an endocrine organ that releases αCGRP and supports acutely the increase in cardiac output. In addition, as we show here, this hormonal signal might aid the heart to discern exercise from other loads and provoke the physiological phenotype of cardiac hypertrophy. Thus, CGRP might need to be added to the continuously growing list of myokins.

## Materials and Methods

*Animals*

Mice globally deficient for αCGRP were kindly provided by Ron B. Emeson (Lu et al., 1999). Mice transgenic for the calcitonin receptor-like receptor (CLR-tg) overexpress a V5 (GKPIPNPLLGDST) tagged rat CLR under control of the mouse smooth-muscle α-actin promoter (Kunz et al., 2007). C57BL6 (BL6) matched to the $\alpha CGRP^{-/-}$ mice and non-transgenic littermates (BL6xDBA2) of CLR-tg mice served as respective wild type control groups or were used for the training experiments. Male mice (except for the measurement of spontaneous activity and body fat content) at an age between 12 and 16 weeks were used for all experiments. The experiments in this study conformed to the 'European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes' (Council of Europe No 123, Strasbourg 1985) as well as institutional and local governmental guidelines.

*Surgery*

Telemetric blood pressure sensors (TA11PA-C10, DataSciences International) were implanted as described (Schuler et al., 2010) to enable continuous recording of arterial blood pressure and heart rate during the exercise experiments. Anesthetized mice were equipped with a femoral artery catheter and allowed to recover from anesthesia for 15 min for determination of arterial blood gases and blood volume as described (Schuler et al., 2010). Lower legs and hearts of mice, some perfusion fixed with paraformaldehyde (PFA) in PBS, were harvested for CGRP receptor autoradiography (unfixed), HE staining (4% PFA), muscle and capillary ATPase histochemistry (2% PFA) and succinatedehydrogenase staining (unfixed) as described (Riddle et al., 1993, Rosenblatt et al., 1987).

*Exercise tests*

Exercise tests were conducted as described with continuous monitoring of oxygen consumption and carbon dioxide production for $VO_{2max}$ and respiratory exchange ratio (RER) determination (Schuler et al., 2010). Ten minutes prior to these tests, mice were injected s.c. either with 200µl PBS (control) or 20nmol CGRP(8-37) (Bachem) dissolved in 200µl PBS.

In addition, 14 C57BL6 mice were subjected to 3-weeks treadmill exercise with five weekly 45-min units. The training program was initiated at treadmill speed of 16 m/min and inclination of 5° and increased weekly to maximum values of 20 m/min and 9°. Fifteen minutes before each training session half of the mice were injected with 20nmol CGRP(8-37) dissolved in 200µl PBS and the other half with 200µl PBS.

Before and after the training period magnetic resonance imaging (Bruker PharmaScan 47/16) of the heart was performed. A cine FLASH sequence was acquired (TE/TR = 1.8/57 ms, an RF pulse angle $\alpha = 10°$) using the self-gating technique IntraGate (ParaVision 5.0, Bruker BioSpin). Eight contiguous 1-mm thick slices were acquired in short axis orientation over the

entire heart with a spatial resolution of 98 x 98 $\mu m^2$. Time points of maximal and minimal ventricular dimensions defined endsystolic and enddiastolic ventricle volume respectively.

*Heart analysis*

Cardiac hypertrophy in the genetically modified mice was assessed by three different methods i.e. determination of heart index (heart weight to body weight or tibia length ratio), cross sectional areas of cardiac myocytes, and volume of isolated, PFA-fixed cardiac myocytes. To this end cardiac myocytes displaying central nuclei in H&E stained sections were selected for measurement of the cross sectional area with an image analyzing system (MCID 7.0, Ontario). The volume of about 2000 freshly isolated cardiac myocytes (cf. next paragraph), which had been fixed immediately by adding an equal volume of 4% PFA in PBS was determined with a Coulter counter (Beckman Z2) equipped with a 200µm aperture.

Cardiac phenotype was assessed with cDNA obtained from RNA extracts (RNeasy, Qiagen) after Proteinase K (Promega) and on-membrane DNase I digestion (Qiagen)) of the whole heart by droplet digital PCR (ddPCR, Bio-Rad) according to the manufactures instruction. To this end, using the cDNA end-point PCR with always 41 cycles were performed after splitting each sample into 12000 - 15000 droplets. Next the percentage of positive droplets was determined and used to calculate the copy number per µl. All primer / probe pairs used produced less than 1% of intermediate droplets suggesting high specificity. As negative control transcription reactions of isolated RNA were also performed without reverse transcriptase. In addition, each primer / probe pair was tested on genomic mouse DNA and under these conditions none of them showed positive droplets. As pathological heart hypertrophy is characterized by an increased ratio of the Myh6 (α-myosin heavy chain) to Myh7 (β-myosin heavy chain) expression, increased Nppa (natriuretic peptide type A) expression and increased collagen IIIa to collagen IaIb expression ratio ddPCR reactions were conducted using mouse “best coverage” TaqMan® kits from Applied Biosystems for Myh6, Myh7, Nppa, collagen IaIb and collagen IIIa (cf. Table 1). Abundance of Nppa amplicons was normalized with calsequestrin amplicons as the expression of this gene remains

unaffected in non-failing as well as failing hearts (Hullin et al., 1999). RNA quality was assessed before transcribing it to cDNA (iScript, Bio-Rad) by gel analysis and measurement of the A260/A280 ratio. Only samples with a band intensity ratio of the 28S/18S ribosomal RNA >1.5 and a A260/A280 ratio > 1.95 were accepted.

Collagen protein content of the hearts was assed on formalin-fixed paraffin-embedded sections according to a previously published protocol (Lopez-De Leon and Rojkind, 1985). Briefly, using a commercially available kit (Sirius red/Fast green Collagen Staining kit, Chondrex) sections were stained simultaneously for total protein (Fast Green) and collagen (Picrosirius Red). The dye was subsequently eluted from the section and the optical density (OD) of the eluate was read at 540 and 605 nm using a spectrophotometer. The amount of collagen per total protein was calculated as Col/prot = $((OD_{540} - (OD_{605} * 0.291)) / 0.0378) / (OD_{605} / 0.00204)$ according to the manufacturer's instructions.

Finally, heart muscle tissue samples were processed for electron microscopy by fixation in a 6.25% solution of glutaraldehyde as previously described (Hoppeler et al., 1973). Capillary number, fiber number and fiber cross-sectional area were estimated at a final magnification of 1500. Five micrographs per block (20 micrographs per sample) were taken in consecutive frames of slotted grids (type R, 100 A, Veco Co, Amsterdam, The Netherlands) yielding >100 muscle profiles for analysis in each sample. A magnification of 24,000 was used for estimation of the volumes of mitochondria per unit volume of muscle fiber as described (Hoppeler et al., 1973). Ten micrographs per block were taken and projected and fitted with quadratic line grids. Point counting was performed with an A 100 grid (100 test points) for the lower magnification and with a B 36 grid (144 test points) for the higher magnification (Hoppeler et al., 1973). Estimates of variables were obtained according to standard stereological procedures.

*Cell culture*

Adult murine cardiac myocytes were isolated as described (Kabaeva et al., 2008) and cultured for 3.5 days with or without 250nMol αCGRP or 250nMol calcitonin (Sigma). At the end of this cultivation cells were fixed and analyzed for their volume as described above.

*Cardiac αCGRP binding sites and αCGRP expression*

αCGRP binding sites in atria and ventricles of the genetically modified mice were determined by receptor autoradiography using $^{125}$I labeled αCGRP (PerkinElmer) as described (Henke et al., 1985). Adjacent sections were used for immunautoradiography against CGRP (primary antibody rabbit anti-CGRP IgG: 1:2000, Chemicon; secondary antibody: $^{125}$I labeled anti-rabbit IgG, PerkinElmer: specific activity adjusted to 60nCi per slide) as described (Zeller et al., 1995). Unspecific binding was assessed by pre-incubation of the sections with 1μM unlabelled αCGRP and omission of the primary antibody, respectively.

*Muscle analysis*

Capillary contacts and percentage of type II fibers in the gastrocnemius and anterior tibial muscle (TA) were measured according to Rosenblatt et al. (Rosenblatt et al., 1987). Oxidative fibers were also visualized as described (Riddle et al., 1993).

*Estimate of adipose and lean tissue weight*

The ratio of lean to fat body mass was determined using computer tomography at a resolution of 100μm per pixel and slice distance of 1mm acquired throughout the whole mouse body as described (Hillebrand et al., 2010).

*Measurement of spontaneous activity*

Home cage activity was measured in pair-housed mice (pairing always the same genotype, 4 x two mice of CLR-tg, αCGRP-ko, BL6xDBA2 and BL6 each). All test trials started at 3 pm (lights on) and were digitally recorded for 24h with infrared sensitive cameras in the absence

of a human observer. The recorded 24h video material was analysed using the ObserverXT® software (Noldus, Wageningen, Netherlands) (Jirkof et al., 2012).

*Statistics*

All data were analyzed with Excel or the GraphPad PRISM 4 Software (version 4.01) using ANOVA and two-sided Students t-test or non-parametric Kruskal-Wallis test in case the numbers of values were not equal for all groups and with Bonferroni's or Dunn's post hoc test respectively. P values below 0.05 were considered significant.

**Results**

*αCGRP deficiency and CLR overexpression do not affect blood parameters, basal metabolic and cardio vascular parameters, heart muscle composition, skeletal muscle microanatomy, body fat content and spontaneous activity.*

No differences in resting acid base status and, more importantly, blood volume or total hemoglobin were detected between the different mouse lines (Table 2). Basal oxygen consumption, respiratory exchange ratio (RER), mean arterial blood pressure or heart rate were also indistinguishable among the animals used for the exercise tests (Table 3, cf. also to Fig. 1). No differences between the investigated mouse lines could be detected regarding the volume density of mitochondria or myofibrils in cardiac myocytes (Table 4). Whereas skeletal muscle fiber composition and capillary contacts remained unaffected by the genetic manipulations and yielded similar values compared to previous data (Gassmann et al., 2008) (Table 4) body fat content was lower in $\alpha CGRP^{-/-}$ and BL6 animals compared to CLR-tg and BL6xDBA2 controls (Table 5). This greater degree of leanness in $\alpha CGRP^{-/-}$ and BL6 mice might result from their approximately 30% higher spontaneous activity (Table 5). As αCGRP deficiency or CLR overexpression did not affect spontaneous activity or body fat content compared to the respective wt control (Table 5), the greater degree of leanness in $CGRP^{-/-}$ mice apparently results from the genetic BL6 background.

*αCGRP deficiency is associated with cardiac hypotrophy whereas CLR overexpression results in cardiac hypertrophy.*

Despite exhibiting the same spontaneous activity, heart indices (heart weight normalized to body weight or tibia length) were, relative to the respective wt control, significantly reduced in $\alpha CGRP^{-/-}$ mice and elevated in CLR-tg mice (Table 4). Accordingly, $\alpha CGRP^{-/-}$ mice had significantly smaller and CLR-tg mice significantly larger cardiac myocyte volumes as well as cellular cross sectional areas (Table 4).

*Spontaneous exercise performance is reduced in $\alpha CGRP^{-/-}$ mice and increased in CLR-tg mice.*

Compared to their respective wild type controls maximal oxygen consumption ($VO_{2max}$) was 7% lower in $\alpha CGRP^{-/-}$ mice and 13% higher in CLR-tg mice. Moreover, treatment of BL6xDBA2with the αCGRP antagonist CGRP(8-37) decreased their $VO_{2max}$ by 7% (Fig. 1a). Due to some post-surgical problems only two CLR-tg mice could be measured after pretreatment with CGRP(8-37) but in these mice $VO_{2max}$ (151 ±3.96 ml/min/kg) as well as time to exhaustion (TTE; 68 ±6.88 min) was also lower compared to PBS pretreated CLR-tg mice ($VO_{2max}$: 159 ±7.02 ml/min/kg and TTE: 81 ±10.65 min, cf. Fig. 1a & 1b). TTE measured one day later was shortened by 70% in $\alpha CGRP^{-/-}$ and prolonged by 45% in CLR-tg mice compared to the respective control mice. Again, treatment of BL6xDBA2mice with CGRP(8-37) reduced TTE in this case about by 34% (Fig. 1b). At $VO_{2max}$ respiratory exchange ratio was the same in all animals suggesting equal exhaustion in all groups (Fig. 1c).

Compared to BL6xDBA2 control mice, mean arterial blood pressure (MAP) at $VO_{2max}$ was highest in CLR-tg mice whereas BL6xDBA2 mice pretreated with CGRP(8-37) displayed lower MAP values at $VO_{2max}$ (Fig. 1d). Heart rates did not differ between BL6xDBA2, BL6xDBA2 treated with CGRP(8-37) and CLR-tg. In contrast to the unaltered MAP at

$VO_{2max}$, heart rate was suppressed in $\alpha CGRP^{-/-}$ mice compared to their control group (BL6, Fig. 1d & 1e), which fits to the observation of Lu *et al.* made previously in a simple swimming test (Lu et al., 1999). Compared to BL6xDBA2 controls, at $VO_{2max}$ $O_2$-pulse was higher in CLR-tg and reduced in BL6xDBA2 after treatment with CGRP(8-37) whereas the rate pressure product was higher in CLR-tg mice (Fig. 1f & 1g).

*αCGRP signaling promotes cardiac hypertrophy.*

In line with previous reports (Bell et al., 1997, Bell et al., 1995) cultured cardiac myocytes of adult BL6 acquired 11% larger volumes within 3.5 days when 250nmol αCGRP were added to the medium (final concentration: 25nM). In contrast, culturing cardiac myocytes for the same time with 250nmol calcitonin (final concentration: 25nM), a related peptide without affinity to CGRP receptors had no effect on their size (Fig. 2a & 2b).

Endurance training of BL6 mice doubled TTE in both groups irrespective of treatment with PBS or CGRP(8-37) prior to each training session (data not shown). However, the cross sectional area of the cardiac myocytes was significantly smaller in CGRP(8-37) treated animals (Fig. 2c) relative to PBS injected controls. Accordingly, in the latter cohort of animals endurance training triggered significant enlargement (12%) of the ventricular muscle volume whereas it was unchanged in CGRP(8-37) treated animals (Fig. 2d). In addition, the remodeling index (ventricular muscle / enddiastolic volume (De Castro et al., 2007)) was significantly decreased in CGRP(8-37) but nearly unchanged in PBS treated mice when comparing pre- and post-training measures (Fig. 2e).

*Myocardial αCGRP binding sites are increased in CLR-tg mice.*

Using autoradiography and quantitative image analysis we found, in line with others (Sigrist et al., 1986, Franco-Cereceda et al., 1987, Mulderry et al., 1985), more CGRP and αCGRP binding sites in atria compared to ventricles (Fig. 3). Importantly, CLR-tg atria and ventricles displayed significantly more αCGRP binding sites than those of BL6xDBA2 suggesting

CLR-tg hearts to be more sensitive to systemic αCGRP than hearts of wt littermates. BL6 mice and αCGRP$^{-/-}$ mice did not differ in atrial $^{125}$I-αCGRP binding sites while ventricles of αCGRP$^{-/-}$ mice showed less binding (Fig. 3a). Moreover cardiac CGRP expression was the same in all mouse lines except for αCGRP$^{-/-}$ mice that showed, as expected, CGRP immunoreactivity close to background staining (Fig. 3b).

*Myocardial gene expression profile of αCGRP$^{-/-}$ mice resembles a mild pathological phenotype.*

Pathological hypertrophy is associated with re-expression of fetal genes e.g. a down-regulated transcription of myh6 (α-myosin heavy chain) together with an up-regulated transcription of myh7 (β-myosin heavy chain) and Nppa (natriuretic peptide type A) (Perrino et al., 2006). Compared to respective BL6 controls αCGRP$^{-/-}$ mice exhibited significantly increased Myh7/Myh6 expression ratio along with an intensified Nppa expression. In contrast, among CLR-tg heart transcript pools the myh7/myh6 expression ratio and Nppa expression tended to be reduced compared to wt mice (Fig. 4a). Thus, these data suggest a fetal re-programming of the general gene expression in αCGRP$^{-/-}$ hearts that accompanies the emergence of a pathological phenotype. In addition, we measured the collagen III to collagen I expression ratio that is also affected by cardiac stress. These measurements revealed a more than 2-fold increased collagen III/I expression ratio in the CGRP$^{-/-}$ mice with unchanged total (collagen I & III) expression. This expression pattern resembles a beginning pathological cardiac remodeling (Weber et al., 1993). In contrast, CLR-tg mice displayed no different collagen expression compared to their respective wt control (Fig. 4a).

We also looked at the mRNA expression of these three marker genes in the hearts of the BL6 mice subjected to 3 weeks of endurance training. Compared to BL6 mice treated with PBS, the Myh7/Myh6 expression ratio and Nppa expression were found not to be affected in mice treated with CGRP(8-37) prior to each training session although these markers were slightly elevated in CGRP(8-37) treated animals (Fig. 4b). In addition, we found lower total collagen

expression (collagen III + I) when pooling all trained mice and compared them to all pooled sedentary mice (29.1 ±12.5 vs. 47.6 ±6.3 copies/µl, $p < 0.01$). Regarding the peptide treatment there was no significant differences of collagen III/I expression ratio between the trained groups or the sedentary groups (Fig. 4b).

Quantification of Picrosirius Red staining revealed no significant differences between the groups (data not shown).

**Discussion**

The present study sheds light on the ongoing debate regarding the adaptations of the heart to various stresses, such as exercise or hypertension, which trigger either physiological or pathological hypertrophy. Using either mice lacking αCGRP, mice overexpressing αCGRP-receptors in the heart (CLR-tg) or treating mice with the specific αCGRP-receptor blocker CGRP(8-37) we show that αCGRP is a crucial regulator of maximum exercise capacity. These observations cannot be explained by different spontaneous activities, lean to fat body mass ratios, blood volumes, and muscle capillarization or fiber composition as neither αCGRP deficiency nor CLR overexpression affected these parameters. However, heart indices and myocyte volumes were decreased in $\alpha CGRP^{-/-}$ mice and increased in CLR-tg mice. Interestingly, $\alpha CGRP^{-/-}$ mice but not CLR-tg animals showed a fetal reprogramming expression profile in resemblance of a maladaptive cardiac phenotype (Perrino et al., 2006). In line with our *in vitro* findings whereby incubation with αCGRP resulted in significantly larger cardiac myocytes as seen by others (Bell et al., 1997, Bell et al., 1995), exercise-induced heart hypertrophy in BL6 mice was abrogated by CGRP(8-37) treatment. Thus, αCGRP augments maximum exercise capacity not only by acutely triggering positive chronotropy and inotropy (Fisher et al., 1983, Ishikawa et al., 1988, Huang et al., 1999, Kunz et al., 2007) but also because the peptide appears to be the hormonal signal that enables the heart to distinguish physiological from pathological stresses.

The long-standing hypothesis that chronic cardiac stresses such as hypertension, stenosis of the outflow tract and others induce pathological heart adaptation whereas exercise, as intermittent stress, results in physiological heart remodeling has recently been questioned when Perrino et al. (Perrino et al., 2006) demonstrated that in contrast to endurance training, intermittent aortic constriction of the same time scheme resulted in pathological hypertrophy. In addition, the Myh7/Myh6 expression ratio and Nppa expression indicating a maladaptive cardiac phenotype was massively and significantly increased by chronic but only marginally by intermittent transverse aortic constriction (Perrino et al., 2006). Accordingly, the adult $\alpha$CGRP$^{-/-}$ mice in our study displayed a slight although significant increase of the Myh7/Myh6 expression ratio as well as Nppa expression. Other biomarkers of pathological heart hypertrophy include cardiac fibrosis with increased collagen expression as well as altered relative expression of collagen III and I. However, the collagen III/I ratio is further known to change according to type, intensity and duration of the pathology, and changes with time. Typically, a higher collagen I expression occurs during more severe and longer lasting pathologies, whereas higher relative collagen III expression results when the pathology is mild and of short duration (Eleftheriades et al., 1993, Carver et al., 1991, Weber et al., 1988). Moreover, increased collagen deposition, although to a much lower degree, is also found in exercise induced, physiological heart adaptation, again, in dependence of duration and intensity of the training protocol (Lindsay and Dunn, 2007, Eleftheriades et al., 1993, Guimaraes et al., 2012). Thus, this multiparametric impact on collagen synthesis and ratios makes moderately increased collagen expression quite difficult to interpret in regard to the type of cardiac adaptation. With these cautionary notes in mind, the observed ~2-fold increased collagen III/I expression ratio in the CGRP-/- mice with unchanged total collagen (I + III) expression might indicate the beginning of a pathological cardiac remodeling process (Weber et al., 1993). Thus, cardiac adaptation in mice challenged by a lifelong $\alpha$CGRP-deficiency ($\alpha$CGRP$^{-/-}$ model) might mimic the course of a mild pathological hypertrophy, in agreement with the reduced spontaneous exercise performance of these animals. Conversely,

bouts of αCGRP released from exercising skeletal muscles might specifically characterize physiological overload situations. Accordingly, the formation of an athlete's heart, e.g. ventricular enlargement at an unchanged or slightly increased remodeling index (ratio between myocardial volume and enddiastolic volume), was observed after a three weeks training period in PBS injected BL6 mice but not in CGRP(8-37) injected BL6 mice. In these latter mice the myocardial volume remained unchanged whereas the remodeling index decreased significantly (Fig. 2d & 2e), which characterizes cardiac maladaptation (De Castro et al., 2007). Unfortunately, assessing cardiac function directly was not possible, Hence, we do not know whether altered diastolic or systolic functions or both accompany the differences in exercise performance or heart geometry observed in the various experimental groups.

Functional αCGRP-receptors in cardiac myocytes of rats or mice have been demonstrated previously (Huang et al., 1999) and consist of the CLR, a seven-transmembrane domain (7TM) protein, and the receptor activity modifying protein 1 (RAMP1) (McLatchie et al., 1998). Despite its atypical heterodimeric composition the αCGRP-receptor shares the common features of most classical 7TM-receptors including G-protein coupled signaling via activation of the adenlyatcyclase or the βγ G-protein dimer (Meens et al., 2011). Interestingly, the αCGRP-receptor interacts also with β-arrestin 2 (Hilairet et al., 2001). β-arrestin signaling promotes cardio-protection in situations of chronic catecholamine or mechanical stresses whereas incessant G-protein dependent signaling is known to confer cardiotoxic effects (Whalen et al., 2010). Indeed, β-arrestin-biased ligands of the $\beta_1$-adrenergic receptor that is always stimulated during cardiac stresses might be cardio-protective and promising for heart failure therapy (Whalen et al., 2010). Of note, there are two types of 7TM G-protein coupled receptors (GPCRs), those forming stable signaling complexes with β-arrestin (class B receptors) and those able to form only transient signaling complexes (class A receptors) (Shenoy and Lefkowitz, 2011). Thus, co-activation of GPCRs from different classes, while recruiting β-arrestins to GPCRs of both types, could yield a preferred binding of arrestin partner proteins by high affinity receptors of the B class. Cardiac αCGRP-receptors might

also recruit β-arrestin to the membrane as it has been shown for numerous 7TM-receptors (DeWire et al., 2007) and this way facilitate shifting signaling of $\beta_1$-adrenergic receptors towards β-arrestin dependent pathways. In line with this β-arrestin recruitment hypothesis is the fact that despite the interaction of β-arrestin with the αCGRP-receptor (Hilairet et al., 2001) the latter lacks the highly conserved β-arrestin binding motifs of other GPCRs (Oakley et al., 2001). Possibly the interaction between αCGRP-receptor and β-arrestin is even weaker than that of class A GPCRs and thus more easily re-direct β-arrestin to class B GPCRs receptors such as the $\beta_1$-adrenergic receptor.

During exercise the mechanical and chemical status of exercising skeletal muscles is continuously monitored (Boushel, 2010) by A∂ and C fibers (Mitchell et al., 1983, Amann et al., 2011). In these nociceptor-like, chemically sensitive fibers Transient Receptor Potential, vanilloid family member 1 channels induce αCGRP release when stimulated e.g. by reduced tissue pH (Kichko and Reeh, 2009, Jonhagen et al., 2006), which in turn might trigger the previously shown exercise-induced rise of plasma αCGRP concentrations (Schifter et al., 1995, Lind et al., 1996, Hasbak et al., 2002). Interestingly, Schifter *et al.* (Schifter et al., 1995) report for the same workload a marginal an inverse correlation between increasing αCGRP plasma levels and training conditions indicating that in well-trained subjects muscular αCGRP release is reduced similar to sympathetic activity (Hautala et al., 2008). Thus, at the same workload muscles of untrained individuals may release more αCGRP or, in other words, exhibit a higher endocrine activity. In this context it should be noted that αCGRP elevates directly, beta-receptor- and sympathetic nervous system-independent atrial force as well as contraction and relaxation speed (Ishikawa et al., 1988). This finding is well in line with data from para- and tetraplegic patients where leg exercise in these individuals increases Q even in the absence of a functional sympathetic nervous system (Dela et al., 2003). Thus, endocrine coupling between exercising skeletal muscles and heart evidently act in parallel to the sympathetic nervous system-dependent metaboreflex. Interestingly, in patients with spinal cord injury leg exercise increased plasma αCGRP levels and this was

more pronounced in tetraplegic than paraplegic patients (Kjaer et al., 2001). To interpret these data one should emphasize that paraplegic patients exhibit residual sympathetic nervous system activity whereas tetraplegic individuals are devoid of it. These observations therefore also suggest the potential of αCGRP to compensate even gradually during exercise for the loss of the sympathetic nervous system.

Another physiological condition associated with a considerable increase of Q also might be of interest in this context. Plasma concentrations of αCGRP increase parallel to its systemic and regional hemodynamic effects during pregnancy (Gangula et al., 2001). In addition, female rodents are more susceptible for the development of physiological heart hypertrophy than males (De Bono et al., 2006, Konhilas et al., 2004). Pregnancy requires a marked increase in Q within a relative short time period to fuel the rapid fetal growth but to also fully maturate the uterine arcade as an essential prerequisite of the high perfusion of the gravid uterus (Gassmann et al., 2008). Indeed, next to exercise, pregnancy is another albeit more chronic non-pathological condition that results in physiological cardiac hypertrophy without a fetal gene reprogramming pattern (Eghbali et al., 2006). Thus, αCGRP appears to maintain also cardiac hypertrophy during pregnancy within the boundaries of a physiological phenotype.

Finally, it is noteworthy that in addition to αCGRP also adrenomedullin binds and activates the CLR when the latter is associated with the receptor activity modifying protein 2 (RAMP2) or RAMP3 instead of RAMP1, which defines the αCGRP specificity of the CLR (McLatchie et al., 1998). Adrenomedullin is essential for embryonic heart development because deletion of either RAMP2 or adrenomedullin results in lethal vascular, lymphatic and cardiac malformations (Fritz-Six et al., 2008, Shindo et al., 2001). On the other hand deletion of αCGRP is not lethal (Lu et al., 1999). Regarding adrenomedullin plasma concentrations in response to exercise data are somewhat conflicting. Some studies found no changes of the adrenomedullin plasma concentration with exercise (Poveda et al., 1998), others a slight although significant increase in plasma adrenomedullin (Hasbak et al., 2002), e.g. at 90 min – but not 30 min – of submaximal exercise (Krzeminski et al., 2006). In our training protocol

one session lasted 45 min. According to the findings of Krzemiński et al. (Krzeminski et al., 2006) this time is most likely too short to result in an increased adrenomedullin plasma concentration. However, the same group also reported an about 50% increase in adrenomedullin plasma concentration with 2x 3min grip exercise (30% of maximal voluntary contraction) (Krzeminski et al., 2002) that activates much less muscle mass than the bicycle ergometer exercise of their later study (Krzeminski et al., 2006). This discrepancy, regarding both the higher total increase in adrenomedullin as well as the much faster response in hand grip exercise, remains unclear. In contrast, data regarding increased CGRP plasma concentration during exercise are much more consistent (Schifter et al., 1995, Lind et al., 1996, Hasbak et al., 2002)

Adrenomedullin is a strong vasodilatator especially in the pulmonary vasculature and it has been shown that adrenomedullin inhalation improves exercise performance in patients suffering from pulmonary hypertension (Nagaya et al., 2004). This effect was however not ascribed to a direct positive inotropic effect of adrenomedullin on the heart but rather to a reduced vascular resistance in the pulmonary circulation, which in turn reduces cardiac afterload, and consequently improves cardiac index (Nagaya et al., 2004). Another study demonstrates an inotropic effect of adrenomedullin in isolated perfused rat hearts (Szokodi et al., 1998). However, the effective dosages in this study were at least 1 to 2 orders of magnitude higher than the plasma concentrations found in man under rest or exercise (Krzeminski et al., 2006, Krzeminski et al., 2002, Hasbak et al., 2002) and at high concentrations adrenomedullin might also activate CGRP receptors (Liao et al., 2013).

In summary, αCGRP appears to be the key hormonal signal that promotes physiological cardiac hypertrophy by allowing the heart to distinguish physiological, exercise-induced from pathological stresses. This also could explain why endurance sport is quite favorable to heart failure patients (Ventura-Clapier, 2009). Although our findings could entice athletes to use αCGRP for improving their endurance capacity, future αCGRP agonists might inhibit in patients suffering from various diseases the emergence of pathological cardiac hypertrophy.

Such a treatment option could be especially beneficial for individuals who cannot do endurance sport.

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**Conflict of interest**

none.

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**Tables**

**Table 1**

TaqMan® Gene Expression Assays used for ddPCR

| Gene name | Gene Symbol | Reference sequence | Assay location | Applied Biosystems reference | Label |
|---|---|---|---|---|---|
| Myosin, heavy polypeptide 6, cardiac muscle, alpha (variants 1 & 2) | Myh6 | http://www.ncbi.nlm.nih.gov/nuccore/NM_001164171.1<br>http://www.ncbi.nlm.nih.gov/nuccore/NM_010856.4 | 2635<br>2571 | Mm00440359_m1 | FAM |
| Myosin, heavy polypeptide 7, cardiac muscle, beta | Myh7 | http://www.ncbi.nlm.nih.gov/nuccore/NM_080728.2 | 5095 | Mm01319006_g1 | FAM |
| Natriuretic peptide type A | Nppa | http://www.ncbi.nlm.nih.gov/nuccore/NM_008725.2 | 209 | Mm01255747_g1 | FAM |
| Calsequestrin 1 | Casq1 | http://www.ncbi.nlm.nih.gov/nuccore/NM_009813.2 | 564 | Mm00486725_m1 | VIC |
| Collagen I | Col1a1 | http://www.ncbi.nlm.nih.gov/nuccore/NM_007742.3 | 4071 | Mm00801666_g1 | FAM |
| Collagen III | Col3a1 | http://www.ncbi.nlm.nih.gov/nuccore/NM_009930.2 | 4460 | Mm01254476_m1 | FAM |

**Table 2**

Blood parameters (male mice)

| | **aCGRP$^{-/-}$** | | **BL6** | | **BL6xDBA2** | | **CLR-tg** | |
|---|---|---|---|---|---|---|---|---|
| | *mean ±SD (n=5)* | | *mean ±SD (n=4)* | | *mean ±SD (n=4)* | | *mean ±SD (n=6)* | |
| *hematocrit (%)* | 46.0 | ±1.1 | 45.9 | ±2.1 | 46.8 | ±1.9 | 47.0 | ±1.7 |
| *hemoglobin (g/dl)* | 15.0 | ±0.3 | 14.9 | ±0.4 | 16.1 | ±1 | 16.0 | ±0.4 |
| *pH* | 7.4 | ±0.04 | 7.3 | ±0.03 | 7.3 | ±0.03 | 7.3 | ±0.03 |
| *$pO_2$ (mmHg)* | 105.1 | ±9.9 | 92.5 | ±10.3 | 94.8 | ±6.8 | 98.7 | ±4.8 |
| *$pCO_2$ (mmHg)* | 44.2 | ±2.8 | 36.8 | ±7 | 33.0 | ±2.8 | 35.4 | ±2 |
| *base excess (mmol/L)* | -0.7 | ±2.1 | -4.3 | ±4 | -7.1 | ±0.4 | -6.7 | ±1.2 |
| *$O_2$ saturation (%)* | 96.7 | ±3 | 92.0 | ±2 | 93.6 | ±1 | 94.7 | ±2 |
| *Blood volume (µl)* | 2667.8 | ±175 | 2551.2 | ±553 | 1871.8 | ±98 | 1915.8 | ±111 |
| *Blood volume (% of body weight)* | 10.3 | ±0.9 | 10.0 | ±1.1 | 9.7 | ±0.2 | 9.8 | ±0.8 |
| *total hemoglobin (g)* | 0.399 | ±0.02 | 0.377 | ±0.08 | 0.301 | ±0.01 | 0.307 | ±0.02 |

**Table 3**

Basal values in awake male mice subsequently used for testing spontaneous exercise performance (cf. Fig. 1)

| | **aCGRP$^{-/-}$** *mean ±SD (n=11)* | **BL6** *mean ±SD (n=9)* | **CLR-tg** *mean ±SD (n=9)* | **BL6xDBA2** *mean ±SD (n=8)* | **BL6xDBA2 +CGRP(8-37)** *mean ±SD (n=8)* |
|---|---|---|---|---|---|
| *Weight (g)* | 24.5 ±2.8 | 26.2 ±1.9 | 26.6 ±1.4 | 26.0 ±1.8 | 26.5 ±1.8 |
| *$VO_2$ (ml/min)* | 50.0 ±3 | 50.6 ±1.8 | 47.6 ±2.4 | 49.0 ±2.3 | 50.0 ±1 |
| *respiratory exchange ratio* | 0.82 ±0.01 | 0.81 ±0.02 | 0.81 ±0.01 | 0.82 ±0.02 | 0.82 ±0.01 |
| *mean arterial blood pressure (mmHg)* | 107.8 ±7.2 | 111.7 ±4.2 | 104.2 ±2.3 | 104.3 ±8.3 | 109.8 ±5.5 |
| *heart rate ($min^{-1}$)* | 510 ±44 | 540 ±37 | 506 ±49 | 509 ±40 | 525 ±37 |

**Table 4**

Morphologic characteristics of skeletal muscle and heart of male mice

| | **aCGRP$^{-/-}$** | | **BL6** | | **CLR-tg** | | **BL6xDBA2** | |
|---|---|---|---|---|---|---|---|---|
| | *mean* | *±SD* | *mean* | *±SD* | *mean* | *±SD* | *mean* | *±SD* |
| Skeletal muscle | | | | | | | | |
| *type II fibers, gastrocnemius (%, ATPase)* | 63.0 | ±13 | 67.0 | ±14 | 74.0 | ±10 | 68.0 | ±10 |
| *oxidative fibers, tibialis ant. (%, SDH)* | 47.0 | ±11 | 43.0 | ±13 | 47.0 | ±14 | 47.2 | ±16 |
| *capillary contacts* | 6.3 | ±1.3 | 6.0 | ±1.4 | 6.1 | ±2.1 | 6.4 | ±1.8 |
| Heart | | | | | | | | |
| *heart index, normalized to body weight (mg/g)* | 4.76 | ±0.3* | 5.64 | ±0.66 | 5.39 | ±0.3* | 4.83 | ±0.3 |
| *heart index, normalized to tibia length (mg/cm)* | 0.067 | ±0.01*** | 0.085 | ±0.01 | 0.080 | ±0.01*** | 0.064 | ±0.01 |
| *cardiac myocyte volume (*$10^4$ *fl)* | 1.18 | ±0.15* | 1.57 | ±0.16 | 2.76 | ±0.63 | 2.26 | ±0.4 |
| *cardiac myocyte cross sectional area (*$10^2$ *µm2)* | 3.45 | ±1.3*** | 4.49 | ±1.8 | 4.95 | ±2.1*** | 3.35 | ±1.6 |
| Cardiac myocytes | | | | | | | | |
| *total mitochondria volume / fiber volume (%)* | 37.39 | ±0.48 | 37.36 | ±1.57 | 36.33 | ±1.34 | 36.43 | ±2.15 |
| *nucleus volume / fiber volume (%)* | 1.38 | ±0.06 | 0.95 | ±0.12 | 0.86 | ±0.22 | 0.38 | ±0.001 |
| *intramyocellular lipid volume / fiber volume (%)* | 0.40 | ±0.13 | 0.28 | ±0.09 | 0.32 | ±0.15 | 0.45 | ±0.07 |
| *myofibril volume / fiber volume (%)* | 55.32 | ±0.1 | 56.50 | ±1.57 | 56.93 | ±2.92 | 57.42 | ±1.33 |
| *remaning fiber volume (%)* | 5.51 | ±0.77 | 4.92 | ±0.21 | 5.56 | ±1.21 | 5.31 | ±0.89 |

*=p<0.05, ***=p<0.001; genetically modified mice compared to their respective wt control
n = 5 for muscle analysis, n = 6 for heart index, n = 4 for other measurements

**Table 5**

Spontaneous activity during 24h of observation and body fat content (females)

| | **aCGRP$^{-/-}$** *mean ±SD* | | **BL6** *mean ±SD* | | **CLR-tg** *mean ±SD* | | **BL6xDBA2** *mean ±SD* | |
|---|---|---|---|---|---|---|---|---|
| *daily activity (h), n = 8* | 14.16 | ±0.85*** | 13.23 | ±0.82** | 9.89 | ±1.37 | 10.93 | ±1.52 |
| *body fat (%), n= 6* | 7.7 | ±2.5*** | 9.6 | ±2.9* | 19.5 | ±5.3 | 19.9 | ±8.6 |

* = p<0.05 vs. BL6xDBA2, ** = p<0.01 vs. BL6xDBA2, *** = p<0.001 vs. CLR-tg

**Legends to figures**

Figure 1: *Spontaneous maximum exercise capacity is decreased in CGRP$^{-/-}$ mice and CGRP(8-37) treated wt mice but increased in CLR-tg mice.*

(a) CLR-tg mice displayed higher and αCGRP$^{-/-}$ mice lower $VO_{2max}$ compared to matched wt controls. Injection of 20nmol of the αCGRP antagonist CGRP(8-37) into BL6xDBA2 prior to the $VO_{2max}$ test decreased their performance (BL6xDBA2 + CGRP(8-37)). (b) Time to exhaustion (TTE) for CLR-tg mice was delayed by approximately 45% while αCGRP$^{-/-}$ mice were already exhausted at about 70% shortened time periods. Accordingly, CGRP(8-37)-treatment reduced TTE in BL6xDBA2 by about 34%. (c) The respiratory exchange ratio measured at $VO_{2max}$ and did not differ between the different experimental groups indicating that all animals reached always the same level of exhaustion at $VO_{2max}$. Compared to their wt controls, mean arterial blood pressure (MAP, d) was higher in CLR-tg mice, lower in BL6xDBA2 mice treated with CGRP(8-37) and unchanged in αCGRP$^{-/-}$ mice whereas heart rate (HR, e) was lower in αCGRP$^{-/-}$ mice only. $O_2$ pulse (f) as a measure for stroke volume and rate pressure product (G) representing myocardial oxygen consumption were altered as $VO_{2max}$ and TTE. Means ±SD of n male mice as indicated on the columns. Kruskal-Wallis test, *** = $p<0.001$ vs. BL6; † = $p<0.05$, †† = $p<0.01$, ††† = $p<0.001$ vs. BL6xDBA2.

Figure 2: *Enhanced αCGRP signaling augments the size of cultured adult cardiac myocytes and is required for development of exercise-induced myocardial hypertrophy.*

(a) Cells cultured for 3.5 days with 250nM αCGRP (gray column) were 11% compared to cells cultured without the peptide (white column). In contrast, cultured ventricular myocytes treated for the same time with 250nM calcitonin (b, gray column) had the same size as cells cultured without calcitonin (white column). Accordingly, cross sectional area (c) as well as myocardial volume (d) was significantly increased by 3 weeks of endurance training with five 45-min units per week in PBS treated mice (black columns) but not altered at all in mice

treated with CGRP(8-37) (white columns). (e) In contrast to PBS treated mice (black column), CGRP(8-37) treated mice (white column) exhibited an 21% increased ($p = 0.076$) ratio between enddiastolic volume (EDV) and myocardial volume (MV) as it is typical for pathological heart hypertrophy (De Castro et al., 2007). Means ±SD of n male mice as indicated on the columns. Students t-test for paired samples, # = $p<0.05$ in (d, e) vs. pre-training, * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$ in (c-e) vs. PBS group.

Figure 3: *Density of αCGRP receptors is lowered in atria of αCGRP$^{-/-}$ mice but elevated in the whole heart of CLR-tg animals.*

Quantitative receptor- (a) and immunautoradiography (b) for CGRP and example autoradiograms of each line (atria marked with arrows heads). Bar graphs indicate 8-bit gray values (0 = black, 255 = white). The right of each double bar represents the data for the atria and the left of each pair represents the ventricles. (a) αCGRP binding was higher in the atria of all lines as evident from the lower gray values. Compared to their wild type controls (BL6xDBA2), CLR-tg had significantly more binding sites in atria as well as ventricles. αCGRP deficient mice had the same density of binding sites in ventricles but significantly less in the atria in comparison to BL6. (b) CGRP immunreactivity was higher in atria and compared with all other lines markedly decreased in αCGRP$^{-/-}$ mice (cf. brighter audiogram). BL6xDBA2, CLR-tg and BL6 did not differ from each other. Means ±SD of 5-6 male mice per group. Students t-test for unpaired samples, * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$.

Figure 4: *αCGRP deficiency promotes a fetal gene expression profile in the heart.*

(a) Copy number ratio of myh7/myh6 and Nppa/calsequestrin in hearts of naïve mice in comparison to matched wild type controls measured with ddPCR. In αCGRP$^{-/-}$ mice the pattern resembles fetal gene expression reprogramming that is typical for pathological hypertrophy with a significant increase of the myh7/myh6 expression ratio and Nppa expression. In contrast, in CLR-tg mice both markers tended to be reduced. (b) Compared to

BL6 mice treated with PBS prior to each training session CGRP(8-37) treatment tended to increase myh7/myh6 expression ratio and Nppa expression (Fig. 4b). In sedentary mice these markers were completely independent of treatment with PBS or CGRP(8-37). Means ±SD of n male mice as indicated on the columns. Students t-test for unpaired samples, * = $p<0.05$.

fig. 1

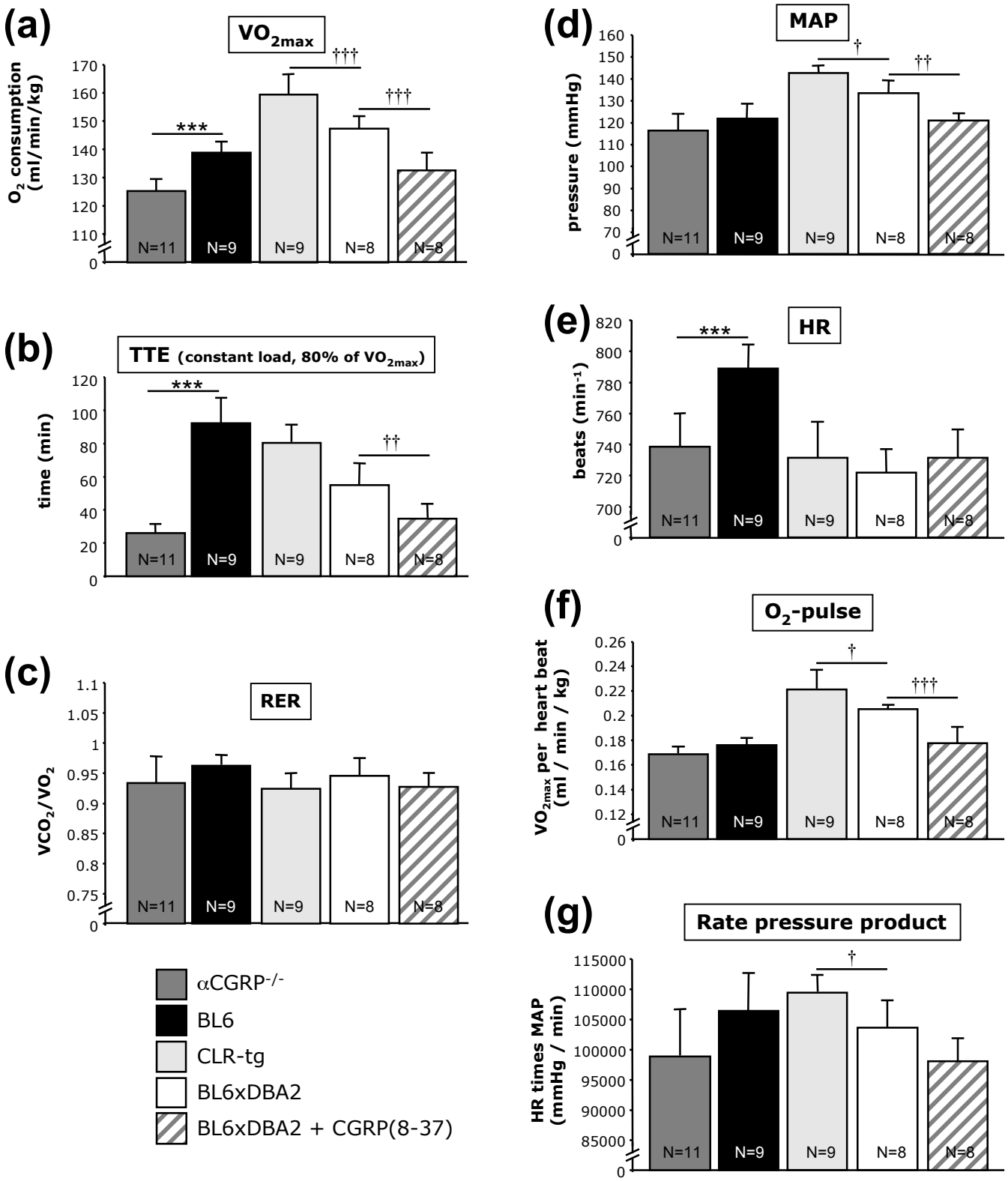

(a)
VO2max
O2 consumption (ml/min/kg)
***
†††
†††
N=11
N=9
N=9
N=8
N=8
(b)
TTE (constant load, 80% of VO2max)
time (min)
***
††
(c)
RER
VCO2/VO2
(d)
MAP
pressure (mmHg)
†
††
(e)
HR
beats (min-1)
***
(f)
O2-pulse
VO2max per heart beat (ml / min / kg)
†
†††
(g)
Rate pressure product
HR times MAP (mmHg / min)
†
αCGRP-/-
BL6
CLR-tg
BL6xDBA2
BL6xDBA2 + CGRP(8-37)

fig. 2

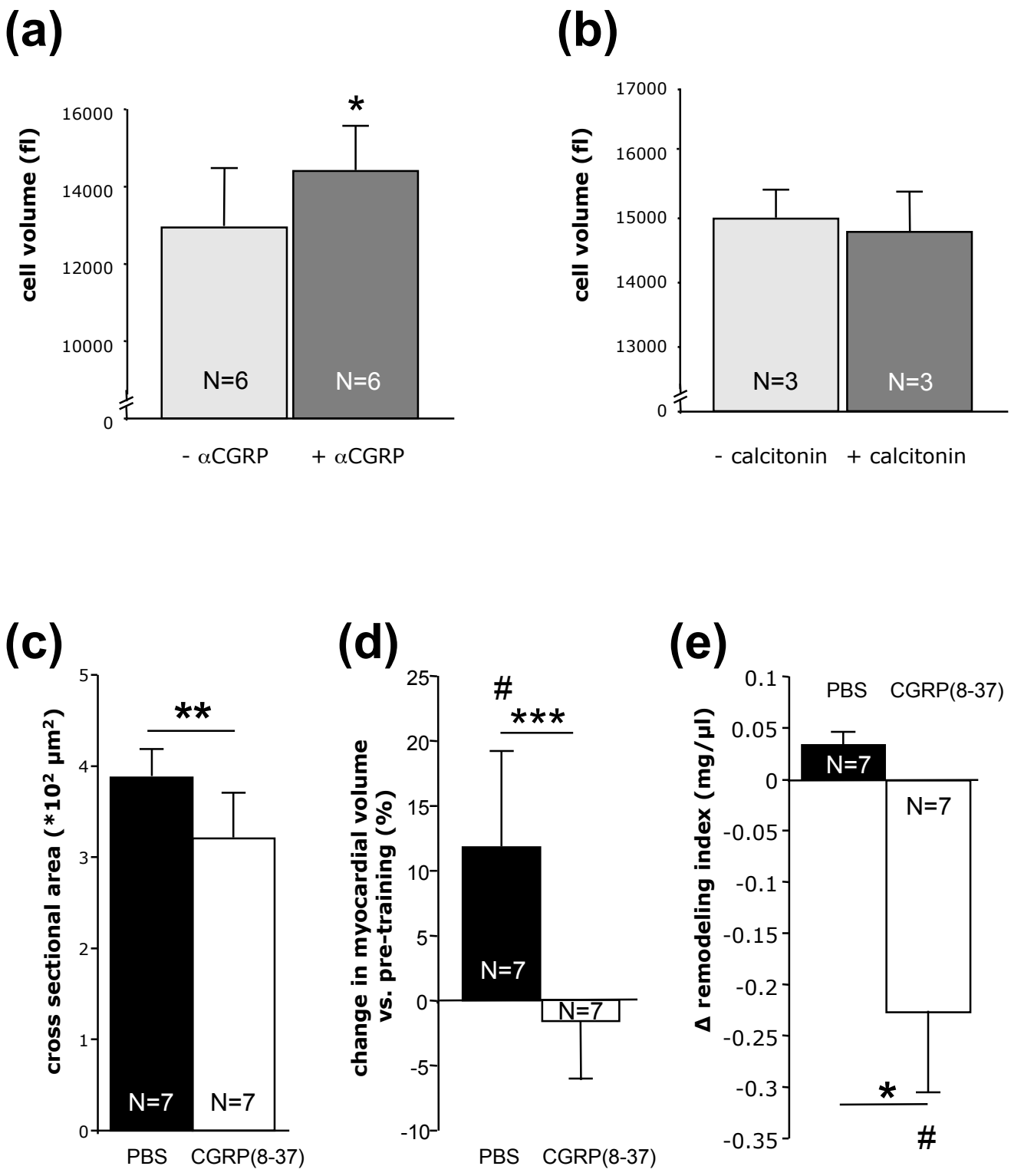
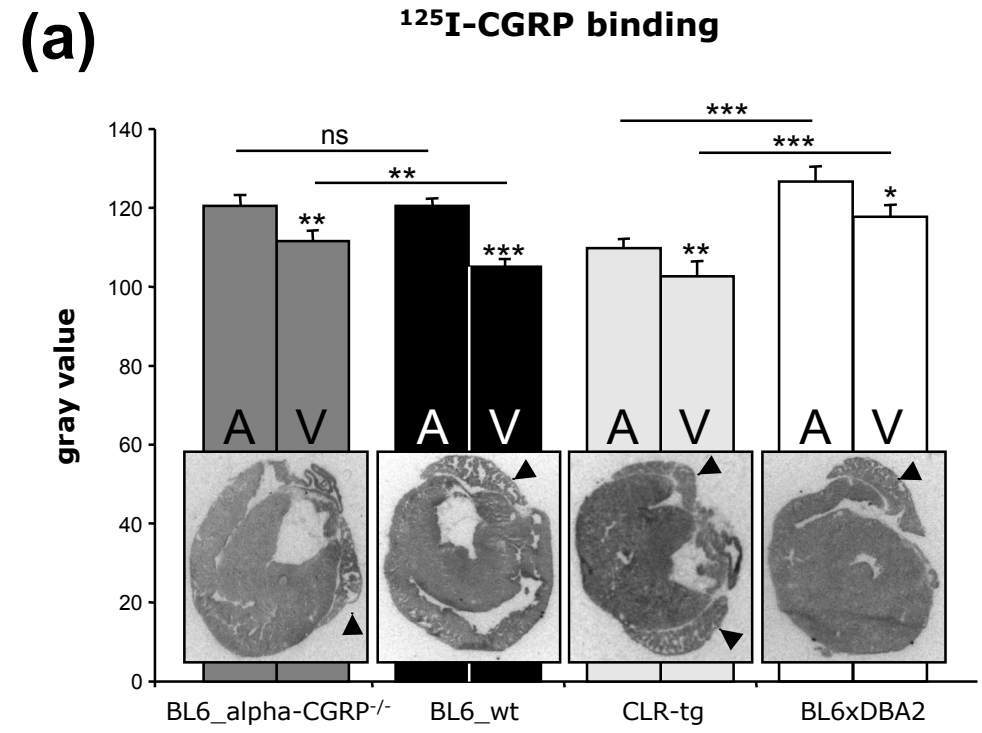

(a)
cell volume (fl)
16000
14000
12000
10000
0
*
N=6
N=6
- αCGRP
+ αCGRP
(b)
cell volume (fl)
17000
16000
15000
14000
13000
0
N=3
N=3
- calcitonin
+ calcitonin
(c)
cross sectional area (*10² µm²)
5
4
3
2
1
0
**
N=7
N=7
PBS
CGRP(8-37)
(d)
change in myocardial volume vs. pre-training (%)
25
20
15
10
5
0
-5
-10
#
***
N=7
N=7
PBS
CGRP(8-37)
(e)
Δ remodeling index (mg/µl)
0.1
0.05
0
-0.05
-0.1
-0.15
-0.2
-0.25
-0.3
-0.35
PBS
CGRP(8-37)
N=7
N=7
*
#

fig. 3


(a)
125I-CGRP binding
gray value
ns
**
***
***
*
**
***
**
A
V
BL6_alpha-CGRP-/-
BL6_wt
CLR-tg
BL6xDBA2

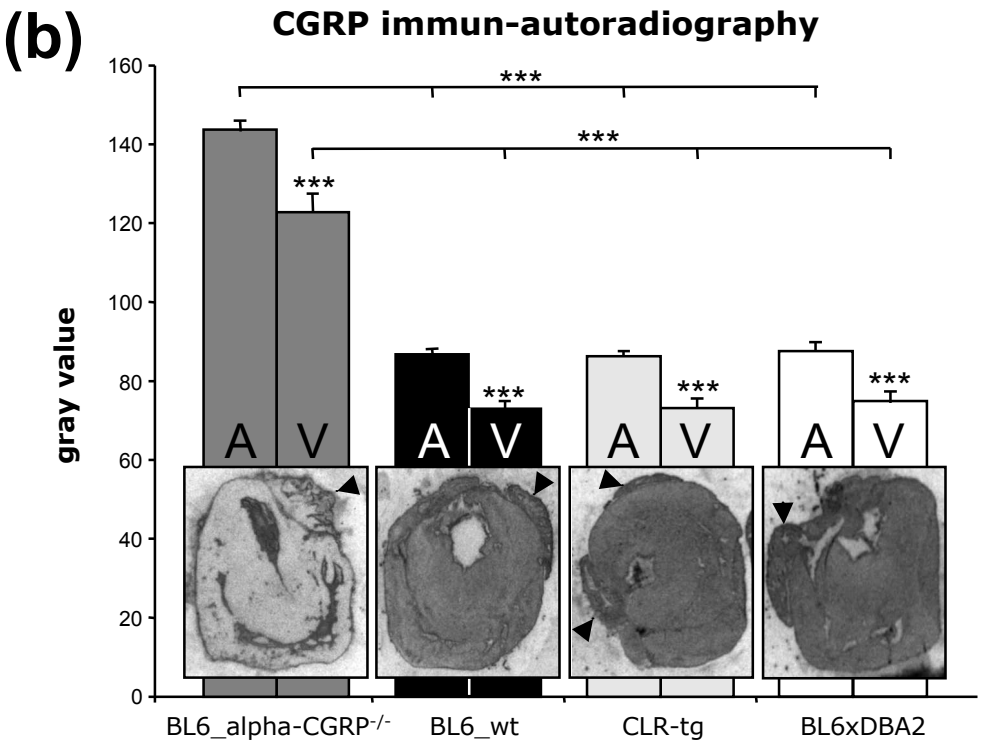
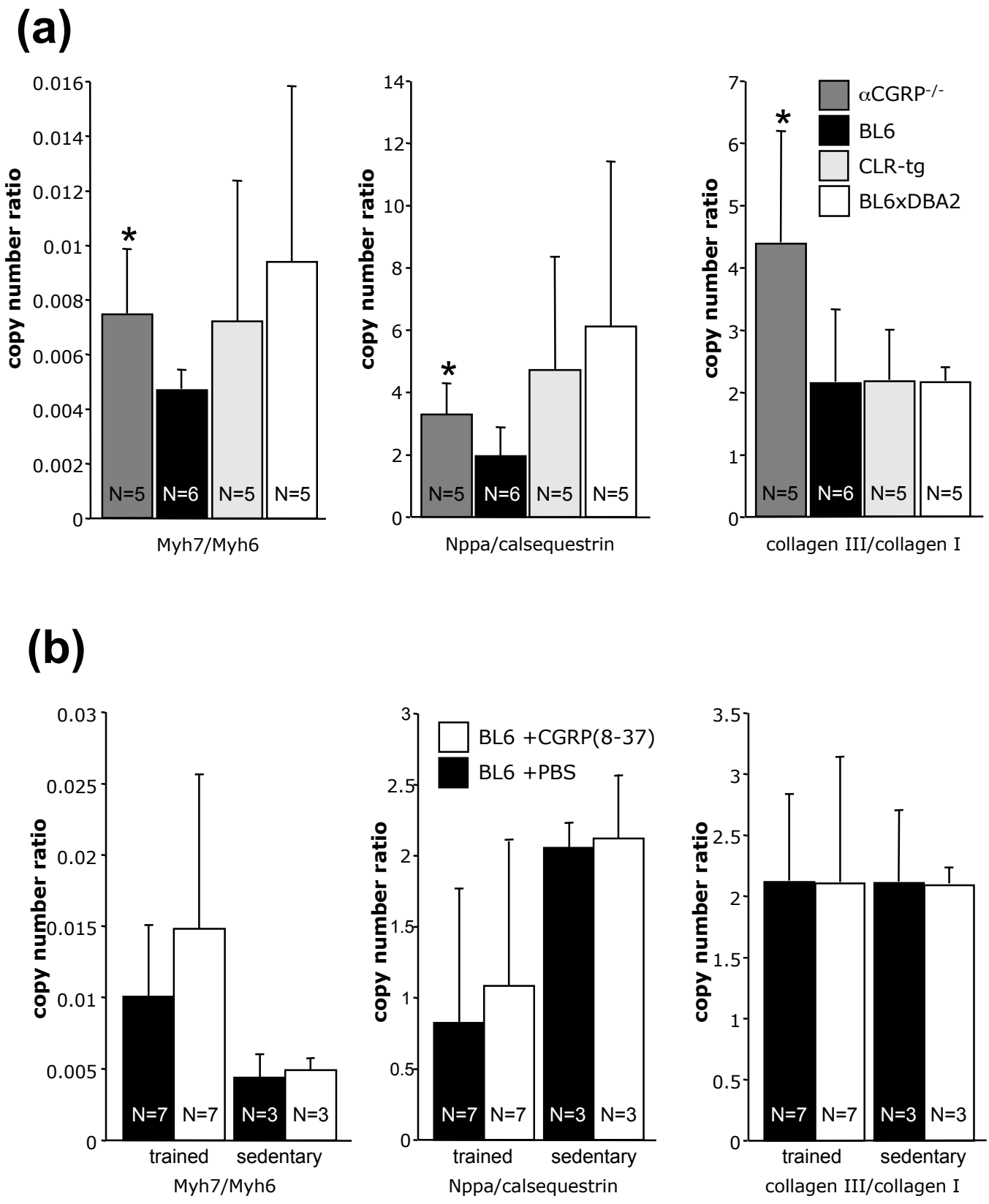

(b)
CGRP immun-autoradiography
gray value
***
***
***
***
***
***
A
V
BL6_alpha-CGRP-/-
BL6_wt
CLR-tg
BL6xDBA2

fig. 4


(a)
copy number ratio
0.016
0.014
0.012
0.01
0.008
0.006
0.004
0.002
0
*
N=5
N=6
N=5
N=5
Myh7/Myh6
14
12
10
8
6
4
2
0
*
N=5
N=6
N=5
N=5
Nppa/calsequestrin
7
6
5
4
3
2
1
0
*
αCGRP-/-
BL6
CLR-tg
BL6xDBA2
N=5
N=6
N=5
N=5
collagen III/collagen I
(b)
copy number ratio
0.03
0.025
0.02
0.015
0.01
0.005
0
N=7
N=7
N=3
N=3
trained
sedentary
Myh7/Myh6
3
2.5
2
1.5
1
0.5
0
BL6 +CGRP(8-37)
BL6 +PBS
N=7
N=7
N=3
N=3
trained
sedentary
Nppa/calsequestrin
3.5
3
2.5
2
1.5
1
0.5
0
N=7
N=7
N=3
N=3
trained
sedentary
collagen III/collagen I